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Visible Light Reception of Accessory Eye in the Giant Snail, *Achatina fulica*, as Revealed by an Electrophysiological Study

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ABSTRACT—Extracellular and intracellular recordings were made in the accessory eye of the giant snail, *Achatina fulica*, in order to compare its photoreceptoral function with that of the main eye. Four types of ERGs were recorded through the suction electrode positioned at various locations in eye preparations. Recordings of the ERG mainly attributable to the accessory eye were accomplished by making fissure on the cornea. Intracellularly two types of receptor potentials with spiking and without spiking were recorded in the accessory and main eyes. With the HRP technique, it became apparent that the receptor potentials without spikes were evoked by photosensory cells type I in both eyes. Spectral sensitivity curve for the accessory and main eyes constructed with the criterion responses of ERGs and receptor potentials were similar. Both have a peak near 480 nm, and are not responsive to light having a wavelength greater than 750 nm.

These results, in combination with morphological data, lead me to conclude that the *Achatina* accessory eye may serve as a monitor of surrounding light intensity in a visible range.

INTRODUCTION

A sensory cell assembly called the accessory retina was first described in the eye of a slug, *Limax maximus*, by Henchman [1]. At present it is well known that such photoreceptoral structures similar to that of *Limax maximus* widely occur in terrestrial slugs [2, 3]. Recently I found a relatively large accessory retina paired with a small lens in the eye of the African giant snail, *Achatina fulica* [4]. Because the *Achatina accessory retina* is histologically discontinuous from the main retina and is invariably paired with a small lens, I called the organ an accessory eye.

As for the function of the slug accessory retina, two hypotheses have been proposed. On the basis of morphological and behavioral observations, Newell *et al.* [2] considered that the accessory retina of *Agriolimax reticulatus* is an infrared light receptor. Mainly from the morphological point of view, Kataoka [3] speculated that the sensory cells of the accessory retina in *Limax flavus* simply monitor the changes in light intensity.

Although a considerable amount of data on the morphology of accessory retinas in pulmonates has been accumulated, there is no information on their electrophysiological properties. The present paper deals with light-elicited electrical activity recorded extra- and intra-cellularly in the accessory eye of *Achatina fulica*. It focuses on the spectral sensitivity of its photosensory cells in comparison with that of sensory cells in the main eye.

MATERIALS AND METHODS

Experimental preparation

Adult Afrian giant snail, *Achatina fulica*, collected in a suburb of Naha, Okinawa, were sent by air and kept in a terrarium. They were fed on lettuce and maintained in cyclic light (12L:12D) at

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room temperature. The eye and its attached stump of optic nerve was cut away from the tentacular tissue under dim red light. The eye-optic nerve preparation was mounted on a small metal clamp in a dish containing a modified Ramsey's snail Ringer solution [5] maintained at approximately 25°C by circulating water at a constant temperature through a built-in glass coil. In such preparations normal electrical responses to photostimulation continued for up to 12 hr.

Electrophysiological recordings

Extracellular recordings were carried out with a glass suction electrode with a tip about 80 μm in diameter. The signal from the electrode were fed into a high input impedance preamplifier (Nihon Koden MEZ-7101). The amplified signals were displayed on a digital storage oscilloscope (Hitachi VC-801L) and read on a chart recorder. The eye-optic nerve preparation obtained by cutting away the skin and musculature was held by sucking with the electrode under dim red light. After the specimen had been prepared, the eye was dark-adapted for at last one hour. Test flashes to evoke and record an electro-retino-gram (ERG) were 1 or 5 sec duration, 1 (−log I) unit intensity and in steps of 50 nm from 400 nm to 800 mn in wavelength.

Intracellular recordings were made with micropipettes filled with 10% horseradish peroxidase (HRP, Sigma type VI) dissolved in 0.5 M KCl, 0.1 M tris buffer (pH 8.6). Electrode resistance was generally 40–80 MΩ. Signals from the electrode were fed into the pre-amplifier, the output of which was displayed on the digital storage oscilloscope or a chart recorder. The microelectrode was inserted into the cells through the opening formed in the cornea by removing the main lens. After the specimens had been prepared, the eye was dark adapted for at least 30 min. Test flashes were of 1 sec duration, 3 (−log I) unit intensity and in steps of 50 nm from 400 nm to 800 nm in wavelength.

Cell identification

After recording the electrical phenomena, HRP was iontophoresed into the cells by introducing depolarizing current pulses (10 nA, 100 msec duration, 5 Hz, 10–20 min). The eye was allowed to diffuse HRP for 60 min and was fixed in 2% glutaraldehyde containing 50 mM cacodylate buffer (pH 7.4) at 4°C. The prefixed sample was rinsed with the same buffer three times and incubated for 30 min at room temperature in the complete reagent containing 0.05% diaminobenzidine (DAB), 0.01% H_2O_2 and the same buffer [6]. The eye was postfixed in 1% OsO_4 for 2 hr, dehydrated, then embedded in Epon. Silver thin sections were contrasted with uranil acetate and lead citrate.

Light stimulation

Light from a 150 W tungsten-bromide lamp (Iwasaki Electric Co.) was focused on the entrance slit of a prism-grating monochromator (Shimadzu RF-503), whose output was conducted to the preparation with a 5 mm diameter optical fiber. The stimulus intensity was controlled with a neutral density circular wedge and neutral density filters. The slit bandpass was 5 nm in the 400 to 600 nm range and 10 nm in the range 600 to 800 nm. The light intensity incident on the preparation was measured with a calibrated vacuum thermocouple (Japan Spectroscopic Co.) and a lock-in amplifier (NF Circuit Design Block LI-574). The maximum intensity, as indicated by 0 (−log I) unit in the figures, was 6.5×10^{10} photons/cm^2. s. A schematic diagram of the experimental arrangement of optical stimulation and electrical recording systems is shown in Figure 1.

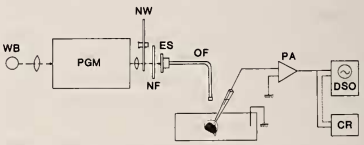


FIG. 1. Experimental arrangement of stimulating and recording systems. CR, chart recorder; DSO, digital storage oscilloscope; ES, electromechanical shutter; NF, neutral density filter; NW, neutral density circular wedge; OF, optical fiber; PGM, prism-grating monochromator; PA, preamplifier; WB, tungsten-bromide lamp.

RESULTS

The morphology of Achatina eye is shown in

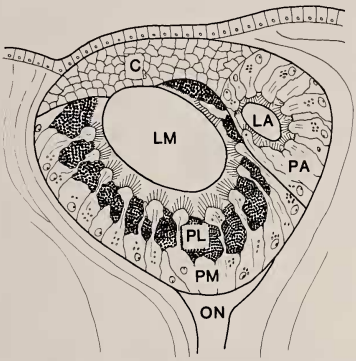


FIG. 2. Schematic drawing of *Achatina* eye. C, cornea; LA, lens of accessory eye; LM, lens of main eye; ON, optic nerve; PA, photosensory cells of accessory eye; PL, pigment cell layer; PM, photosensory cells of main eye.

Figure 2. The eyes of the adult snail are ovoid, about 200 μm wide and 250 μm long, and bear spheroidal main lenses. They are situated near the tips of two retractable ocular tentacles that can be extended nearly 2 cm in length. The accessory eye lies in an extension of the cornea and is always associated with a small lens. Both the main and accessory eyes are composed of two types of photoreceptor cells, type I and type II, and supporting cells. Many of these features have already been described [4]. A marked difference between the morphological appearance of the accessory and main eyes is that the former lacks the pigmented cell layer in the retina. This feature, in addition to the refractivity of the accessory lens, facilitates detection and manipulation of the accessory eye under the stereomicroscope.

General characteristics of electroretinograms

Responses recorded at three sites in the eye are shown in Figure 3. When the electrode was placed on the cornea, the electroretinogram (ERG) exhibited a biphasic negative potential consisting of a rapid fall followed by a slower potential change returning to the baseline (Fig. 3A), as commonly seen in molluscan and annelidan eyes [7–9]. When the electrode was moved to the surface of the accessory eye the response was biphasic, comprising an initial rapid rising followed by a slow negative potential (Fig. 3B). When the electrode was set on the surface of the main eye, the ERG displayed similar waveform to the corneal ERG but had opposite polarity (Fig. 3C).

Except for the differences in amplitude, there were no change in the waveforms of these ERGs with stimulus light in the wavelength range from 400 to 700 nm. No response was evoked in either eye with light having a wavelength greater than 700 nm. From the waveforms of the ERGs and the spatial arrangement of the two kinds of eyes (Fig. 2), it was presumed that the initial rapid positive deflection in the Figure 3B biphasic ERGs originates in the accessory eye and the subsequent gradual negative potential change is due to the main eye. An attempt to eliminate the contribution of the main eye from the biphasic ERG was achieved by making a fissure on the cornea, and ERGs of the accessory eye were recorded (Fig. 3D). Examples of intensity-ERG amplitude double logarithmic plots are shown in Figure 4, where the amplitude data for the two retinas were obtained from ERGs as the difference between the baseline and the positive peak (Fig. 3C and 3D). The largest amplitude recorded on the main eye was about 800 μV, and that of the accessory eye was about 560 μV with the flashes of 1 sec duration. These double logarithmic plots indicate that the intensity-amplitude relation is composed of two linear curves, steep and modrate. The inflection points fell on about 3 ($-\log$ I) unit.

ERG spectral sensitivity

In order to compare the spectral sensitivity of the accessory eye with that of the main eye, a spectral sensitivity curve was constructed. The ERG sensitivity was the relative intensity required to elicit the criterion amplitude of 0.1 mV at each wavelength. Figure 5A shows the spectral sensitivity curves obtained from each of 2 preparations of the main and accessory eye. The spectral sensitivity curves for both eyes exhibited a maximum at about 480 nm and corresponded well to the Dartnall nomograms [10] for a single visual pigment (Fig. 5B).

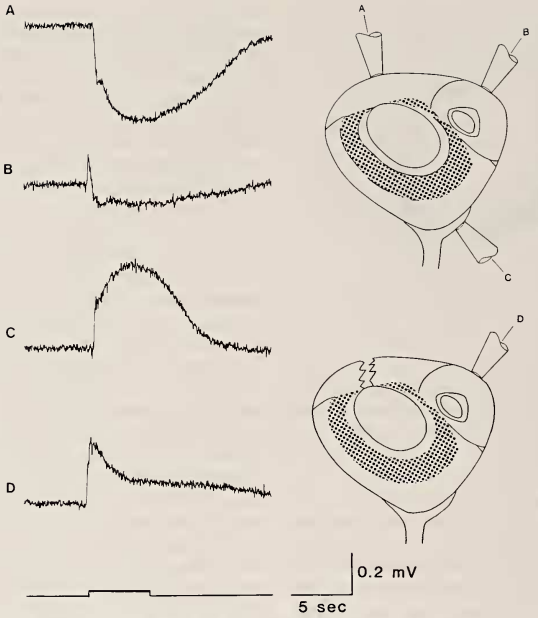


FIG. 3. Electroretinograms recorded through the suction electrode placed at three sites. A, Recording on corneal surface; B, back of accessory eye; C, back of main eye; D, back of accessory eye after making a fissure on the cornea. Trace beneath D indicates the monitor of a 5 sec flash of 500 nm light at 6.5×10^7 photons/cm^2·s ($-\log I = 3$). Right two drawings show their recording sites.

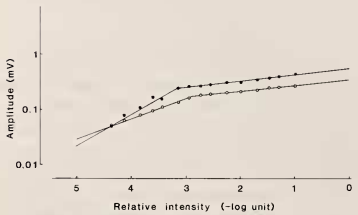


FIG. 4. Double logarithmic plots of ERG amplitude-intensity determined from the mamin eye (closed circles) and the accessory eye (open circles). Stimulus duration was 1 sec of 480 nm light. All data in each curve were recorded from a single preparation.

Intracellular responses

Intracellular recordings from the accessory eye gave two types of light responses (Fig. 6A). The first one was a simple depolarizing receptor potential. The majority of the recordings belonged to this type. These recordings were stable and were obtained from the same cells for up to two hours. The other type was a receptor potential, on which spike burst of the same polarity were superimposed (Fig. 6B). In the figure, the recording was initiated with injury spikes caused by the penetration of the microelectrode. There was only a slight chance of impaling such spiking cells and, in most cases, recordings for these cells failed to be

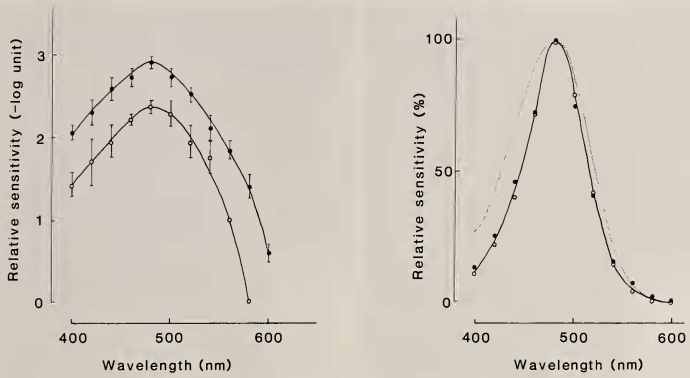


FIG. 5. Spectral sensitivity of the main eye (closed circles) and the accessory eye (open circles). Both curves represent the average of data from 2 preparations of each eye. Each stimulus duration was 1 sec. A, Relative sensitivity is the reciprocal of the light intensity required to elicit a 0.1 mV criterion response. B, Relative sensitivity is plotted in a percentage of the maximum sensitivity in A. Dotted line indicates the Dartnall nomogram whose peak is at 480 nm.

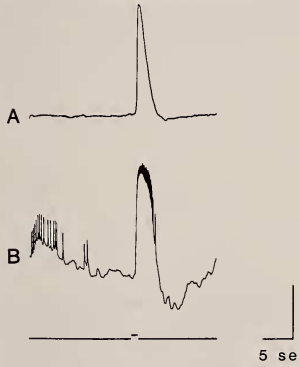


FIG. 6. Intracellularly recorded responses from photosensory cells of the accessory eye. Trace beneath B indicates the monitor of a 1 sec flash. The flash was 480 nm in wavelength and 1 (−log I) unit intensity. A, Receptor potential without spikes. B, Receptor potential superimposed with spike burst. The recording is initiated with injury spikes.

obtained after a few minutes.

The two types of receptor potentials, with identical waveforms to those of the photosensory cells of the accessory eye, were also recorded from the main eye. From the waveform alone it is difficult to tell which eye is evoking the receptor potential. With the HRP technique, the cells with simple receptor potentials were identified as photosensory cell type I in both eyes (Fig. 7). In the dark-adapted state, the resting potentials of photosensory cells in the two kinds of eyes lay between −50 and −60 mV. The potentials of photoinsensitive cells, probably such as corneal and pigment cells, were −70 to −90 mV in both of the eyes.

Intensity-response characteristics of intracellular recordings

Figure 8 shows a sequence of receptor potentials, recorded at different intensities of light, from a type I photosensory cell of the accessory eye. At a high intensity (0, −log I unit), the flash elicits a large depolarizing potential which comes back to the base line through a short plateau and an after hyperpolarization. There was no overshooting in any cases. Attenuating the stimulus intensity (1 or 1.7, −log I unit), the amplitude of the receptor potentials successively decreases, and the repolarizing process turns out to be rapid and monophasic. Figure 9 shows examples of intensity-

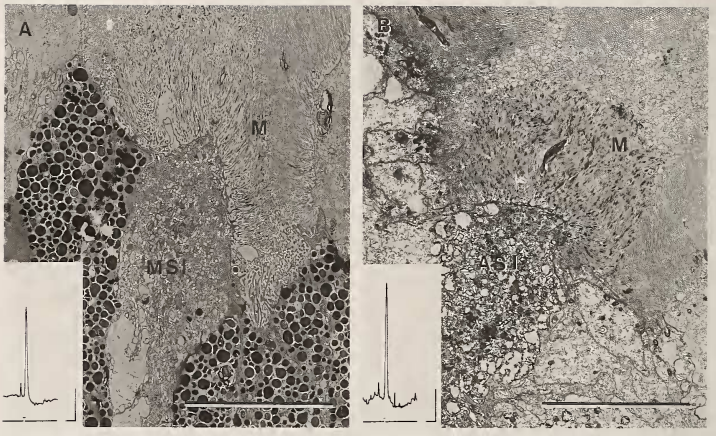
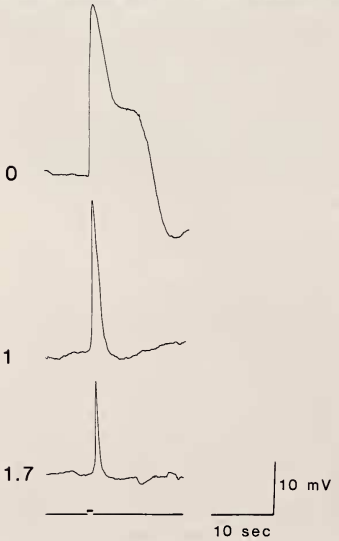


FIG. 7. Electron micrographs of sensory cells type I stained with HRP intracellularly. A, Sensory cell type I in main eye. MSI, sensory cell type 1 in main eye; M, microvilli. B, Sensory cell type I in accessory eye. ASI, sensory cell type 1 in accessory eye; M, microvilli. Calibration bars = 10 μm Each inset shows the receptor potential recorded from the sensory cell shown in the figure. Trace beneath the recording indicates the monitor of a 1 sec flash at 480 nm in wavelength and 3 (−log I) unit intensity, and the calibration bars indicate 5 sec and 10 mV.



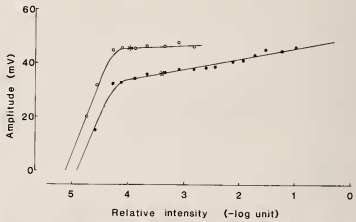


FIG. 9. Amplitude-log intensity curves for type I photosensory cells of the main (closed circles) and accessory (open circles) eyes. Flashes were 1 sec at 480 nm after the sensory cells were fully dark-adapted. All data in each curve were obtained from a single preparation. Asterisks on the curves indicate the point of each standard response respectively (see Results).

FIG. 8. Receptor potentials recorded from type I photosensory cells of the accessory eye at different light intensities. The number to the left of each recording indicates -log relative intensity for that response. Trace beneath the three recording indicates the monitor of a 1 sec flash at 480 nm in wavelength.

amplitude plots measured from type I cells in both of the eyes. The curves seem to show a higher part of the typical sigmoid intensity-response curves [11], where the amplitude of receptor potentials gradually increase as the light intensity increase.

Spectral sensitivity of photosensory cells

The sensitivity was the relative intensity required to evoke a criterion response. The criterion response was obtained as follows: As the intensity of the stimulus light increased, the spectral response curve at a constant photons of stimulus light, whose peak was found at 480 nm, became flat. When the receptor potentials of similar amplitudes (within a deviation range of 5 mV) were recorded in the range over 100 nm (it was from 420 nm to 520 nm), the amplitude at 480 nm was determined as a standard. For instance, the standard response of the samples used in Figure 9 fell on the asterisk in the figure. The criterion response was a half of the standard. Figure 10A shows the spectral sensitivity curve for type I sensory cells in both of the eyes. Open circles show data recorded from the type I cells of the accessory eye and filled circles are from those of the main eye. The sensitivity was also expressed as a percentage of the maximum (Fig. 10B). The data for the curves were obtained from each of 3 preparations of main and accessory eye.

These show that the type I photosensory cells of both eyes exhibit the same spectral sensitivity peaking at 480 nm. The spectral sensitivity curve as well as in the case of the ERGs corresponds well to the Dartnall nomograms [10] for a single visual pigment. Although recordings on the other type of sensory cells, which may be type II sensory cells, were limited in number owing to the technical difficulty described above, the spectral response curves suggest that they may have a similar spectral sensitivity peak to the type I sensory cells. In all cases of both type of cells in the accessory and main eyes, no receptor potentials were evoked by stimulus light having a wavelength greater than 750 nm.

DISCUSSION

Since the accessory photosensory organ in pulmonate came to be known, two contradicting hypotheses (the infrared light receptor and the monitor of light intensity) have been proposed. The present study produced evidence of visible

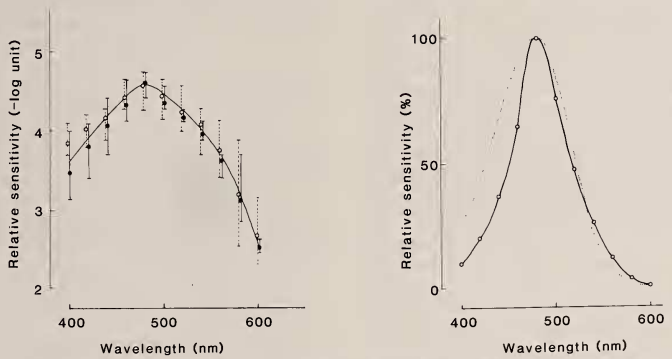


FIG. 10. Spectral sensitivity of type I sensory cells in the main and accessory eyes. Flashes were 1 sec in duration at various wavelengths and intensities. Data were obtained from each of 3 preparations of the main and accessory eye. Open circles are data recorded from the sensory cells of the accessory eye and closed circles are from those of the main eye. A, Relative sensitivity is the reciprocal of the light intensity required to elicit a criterion response (see Results). B, Relative sensitivity is plotted as a percentage of the maximum sensitivity in A. Dotted line indicates the Dartnall nomogram whose peak is at 480 nm.

light reception of the accessory eye by employing an electrophysiological technique. The electrical responses were recorded from the accessory eye with a suction electrode and a microelectrode, and each of them was compared with that from the main eye.

With a suction electrode, the ERGs of the accessory eye which were obtained by lesioning the cornea to convert the bipolar responses into monopolar ones were recorded, and the sensitivity peak was found at a similar wavelength to that of the main eye (480 nm). This sensitivity peaking wavelength was very close to those of other gastropodas' eyes [8, 12–15]. It is unlikely that another peak of sensitivity will be found and the burst of spikes is caused in a range near infrared, since the suction electrode monitored the activity of the accessory eye as a whole in the 400 nm to 800 nm wavelength range.

The intracellular recordings from sensory cells in the accessory and main eyes were made with microelectrodes containing HRP in order to discriminate the activity of the each eye. Satisfactory recordings were obtained from type I sensory cells in both of the eyes. The recordings were used to construct the spectral sensitivity curve, whose sensitivity peak was found at 480 nm and coincided with the sensitivity peak of the ERGs. The other type of sensory cell, which may be sensory cell type II, also responded to visible range light. The accessory eye and the main eye of *Achatina fulica* were composed of only these two types of sensory cells in addition to the supporting cells in the main eye [4]. Two types of ganglion cells identified by some electrical properties may also be included in the main eye [16]. However, these are no longer any sensory cells to receive infrared light. If the accessory eye received infrared light, the main eye could also receive it because of the structural similarity of the sensory cells. Recently, in addition, rhodopsin and retinochrome were found in the accessory photosensory organ of *Limax flavus* [17, 18]. Therfore, in view of these facts, it is concluded that the accessory eye is a visible light receptor having a sensitivity maximum at 480 nm.

Other electrophysiological properties also support the above conclusion. The intensity-amplitude relationships of the ERGs (log-log plots) were represented by two different components in the accessory eye, just as shown in the *Limax* main eye [5] and in the *Achatina* main eye [16]. The two components in the intensity-amplitude relations or in the dark adaptation curve are observed in the cases there are two kinds of photoreceptor cells with different sensitivity such as the cone and rod in vertebrate retina or the sensitivity is changed by the migration of screening pigment granules [19]. In the eye of *Achatina fulica,* pigment granules have been found only in supporting cells [4] and the position of pigment is unaffected by light. Therefore, these two components may also imply the existence of two types of sensory cells with different sensitivity in the accessory eye as well as the similarity between the two eyes.

In the construction of a spectral sensitivity curve, a standard response was adopted in order to obtain a criterion response immediately and to reduce the effects of the intracellular-recording conditions on relative sensitivity in each cell. Although the standard response is a value used tentatively, it was reliable in comparing the relative sensitivity and providing the evidence of visible light reception by the accessory eye.

As one of the other characteristics to advance speculation on the functions of the accessory eye,

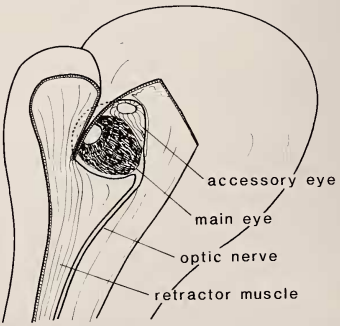


Fig. 11. Schematic diagram of the situation of the accessory and main eyes in a partially retracted optic tentacle. The eyeball was rotated about a right angle from the original situation in a fully extended tentacle.

there is the difference in the location of the two eyes. If the eye preparation is illuminated from a direction other than the pupillary opening of pigment cell layer in the main eye, the main eye may not perceive the illumination, even though the accessory eye perceives it well. Such a condition may occur in a partially retracter optic tentacle (Fig. 11). When a giant snail begins to crawl, it behaves as if it is looking around with the partially retracted optic tentacles (unpubl. data). Similar behavior was also observed in *Agriolimax reticulatus* [2]. In the partially retracted optic tentacles, the eyeball was rotated about a right angle and the pupillary opening of the main eye was masked by epidermal tissues, while the accessory eye might be exposed to environmental light as in the fully extended tentacles. Therefore, the accessory eye may function principally as a luminous intensity meter in the partially retracted tantacle.

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